GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No. G-33-G10 (Q5169-0A0) GTRC/~~GIT~~ XXX DATE 8 / 26 / 85

Project Director: N. Yu School/~~Lab~~ XXX Chem

Sponsor: DHHS/PHS/NIH/NIGMS

Type Agreement: Grant No. 5 R01 GM18894-15

Award Period: From 9/1/85 To 8/31/86 (Performance) 11/31/86 (Reports)

Sponsor Amount:	This Change	Total to Date
Estimated: $		$ 124,478
Funded: $		$ 124,478

Cost Sharing Amount: $ 6,576 Cost Sharing No: G-33-393

Title: Laser Excited Raman Spectroscopy of Biopolymers

ADMINISTRATIVE DATA

OCA Contact John Schonk x4820

1) Sponsor Technical Contact:

James Cassatt
National Institute of Health
NIGMS
Program Administrator
Bethesda, MD 20205
301/496-7175

2) Sponsor Admin/Contractual Matters:

Linda Roberts
National Institute of Health
NIGMS
Grants Management
Bethesda, MD 20205
301/496-7746

Defense Priority Rating: N/A Military Security Classification: N/A

(or) Company/Industrial Proprietary: N/A

RESTRICTIONS

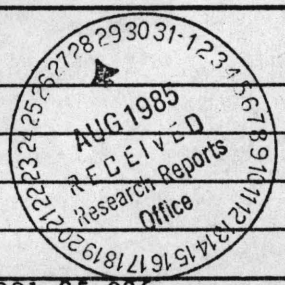
See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

No funds may be expended after 8/31/86.



Continuation of G-33-G09.

SPONSOR'S I. D. NO. 02.108.001.85.026

COPIES TO:

Project Director
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GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 08/24/92

Project No. G-33-G10 Center No. Q5169-0A0

Project Director YU N School/Lab CHEMISTRY

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 3 R01 GM18894-15S1 Contract Entity GTRC

Prime Contract No. _____

Title LASER EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

Effective Completion Date 860831 (Performance) 861130 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other _____	N	

Comments_____

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	N
GTRC	N
Project File	Y
Other _____	N
_____	N

6-33-G10

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER GM 18894-16	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT FROM	THROUGH
NAME OF ORGANIZATION Georgia Institute of Technology	09/01/85	06/24/86

TITLE *(Repeat title shown in item 1 on first page)*
Laser-excited Raman Spectroscopy of Biopolymers

(SEE INSTRUCTIONS)

Publications:

1. Kerr, E. A., Yu, N.-T., Bartnicki, D. F. and Mizukami, H. "Resonance Raman Studies of CO and O_2 Binding to Elephant Myoglobin (Distal His(E7) → Gln)" J. Biol. Chem. 260, 8360 (1985).

2. Kerr, E. A., Yu, N.-T., Gersonde, K., Parish, D. W. and Smith, K. M. "Iron-Histidine Stretching Vibration in the Deoxy State of Insect Hemoglobins with Different O_2 Affinities and Bohr-Effects" J. Biol. Chem., 260, 12665 (1985).

3. Shelnutt, J. A., Alston, K., Ho, Jui-Yuan, Yu, N.-T., Yamamoto, T. and Rifkind, J. M. "Four- and Five-Coordinate Species in Nickel-Reconstituted Hemoglobin and Myoglobin: Raman Identification of the Nickel-Histidine Stretching Mode" Biochemistry, 25, 620 (1986).

4. Gersonde, K., Kerr, E. A., Parish, D. W. and Smith, K. S. "Resonance Raman Investigation of CO-Ligated Monomeric Insect Hemoglobins. Direct Evidence for Reciprocal Changes in Iron-Axial Ligand Bonds Induced by Allosteric Transitions" J. Biol. Chem. (in press).

5. Thompson, H. M., Yu, N.-T. and Gersonde, K. "Resonance Raman Evidence for the mechanism of the Allosteric Control of O_2-Binding in a Cobalt-Substituted Monomeric Insect Hemoglobin" Biophys. J. (in press).

6. Yu, N.-T., Thompson, H. M., Zepke, D. and Gersonde, K. "Mechanism of the Control of Dioxygen Binding in a Dimeric Cobalt-Substituted Insect Hemoglobin. Resonance Raman Evidence for Cobalt-Axial Ligand Bond Changes" Eur. J. Biochem. (in press).

7. Yu, N.-T., Thompson, H. M. and Chang, C. K. "Resonance Raman Studies of Dioxygen and Carbon Monoxide Binding to Imidazole-Appended Hemes" Biophys. J. (in press).

8. Yu, N.-T., Thompson, H. M., Mizukami, H. and Gersonde, K. "The Co-NO Stretching Vibration as a Sensitive Resonance Raman Probe for Distal Histidine-Nitrosyl Interaction in Hemoglobin" Eur. J. Biochem. (in press).

9. Gersonde, K., Yu, N.-T., Kerr, E. A., Smith, K. M. and Parish, D. W. "Heme-Rotational Disorder in Monomeric Allosteric Cyano-Met Insect Hemoglobins Monitored by Resonance Raman Spectroscopy" J. Mol. Biol. (submitted).

Reports

1. General Scientific Goals: No Change.
2. Concise Description of Studies Conducted during the Budget Year, the Results Obtained and their Significance.

(a) Heme-Rotational Disorder in Monomeric Allosteric Cyano-Met Insect Hemoglobins.

The heme-rotational disorder refers to the two heme-rotational components which are related by 180° rotation about the α,γ-meso axis. We have investigated the effect of heme rotational disorder on the resonance Raman spectra of cyanomet CTT III and CTT IV reconstituted with proto-IX, deutero-IX and meso-IX porphyrins. We have identified two Fe-C-N bending modes at 401 cm^{-1} and 422 cm^{-1} (pH 9.5) for ^{57}Fe deutero-IX CTT IV ligated with $^{13}C^{15}N^-$ which are attributed to the two heme-rotational components (Fig. 1 ,excited at 413.1 nm). One Fe-C-N bending mode at 422 cm^{-1} shows a pH-induced shift to 424 cm^{-1} (pH 5.5) indicating the t → r conformational transition, whereas the other bending mode is pH-insensitive representing a non-allosteric component.

Replacing the unsymmetrical porphyrins with the "symmetrical" protoporphyrin-III we eliminate the heme disorder. Sharpening of the Fe-N_ε(His) (at 313 cm^{-1}) and Fe-CN (at 453 cm^{-1}) stretching modes is observed and a single Fe-C-N bending mode (at 412 cm^{-1}) appears (Fig. 2 , excited at 413.1 nm).

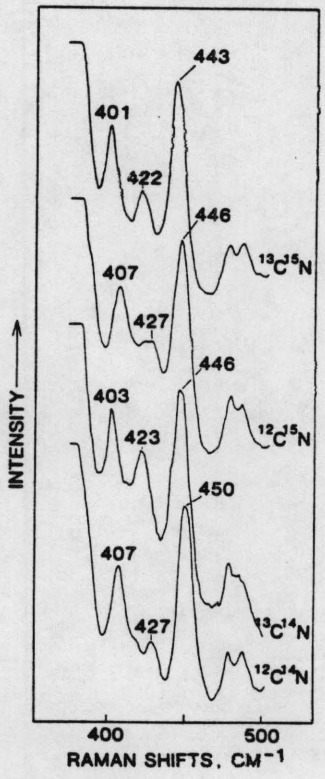


Fig. 1

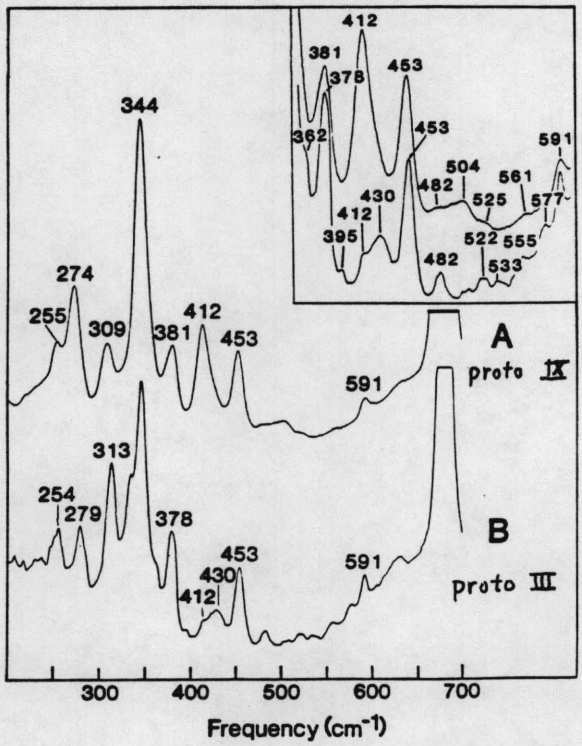


Fig. 2

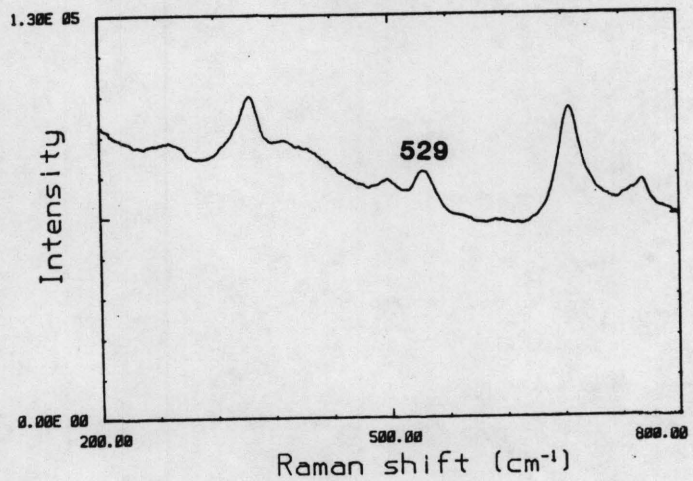
(b) Dioxygen and Carbon Monoxide Binding to Imidazole-Appended Hemes.

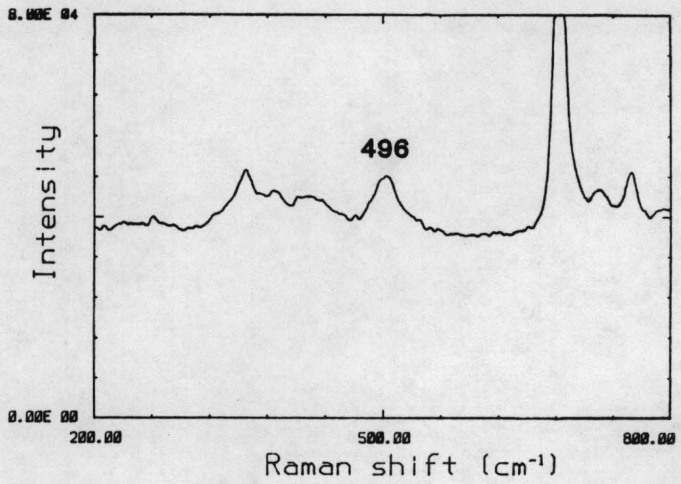
We have employed resonance Raman spectroscopy to probe the effects of proximal base strain on the bonding of O_2 and CO in three synthetic hemins with "Long", "Short", and "Stiff" covalently linked imidazole ligands. The oxy complexes were formed by introducing dioxygen to the deoxy complexes at ~ -70°C. The isotope-sensitive line was detected at 576 cm^{-1} ($^{16}O_2$) in oxy Stiff hemin, which was shifted to 545 cm^{-1} upon $^{18}O_2$ substitution. This is the largest isotope shift (31 cm^{-1}) observed to date, compared to the usual 22-24 cm^{-1}. For the Long and Short hemins, the iron-oxygen associated vibration was detected at 574 and 573 cm^{-1}, respectively. Upon CO binding, we have observed the ν(Fe-CO) stretching frequencies at 497 (Long), 499 (Short) and 496 cm^{-1} (Stiff), somewhat lower than those reported by Mitchell et al. (Inorg. Chem. 1985, 24, 967) for the Traylor's chelated-heme•CO complexes (i.e., 501-506 cm^{-1}).

(c) Surface-Enhanced Resonance Raman Spectroscopy of Metalloporphyrins and Hemoproteins.

We have demonstrated that the Fe-CO stretching vibration in the CO complexes of protoporphyrin IX (in aqueous solution) and sperm whale myoglobin can be detected at ~ 1 μM concentration via surface-enhanced resonance Raman technique. Figs. 3 and 4 exhibit the ν(Fe-CO) modes at 529 cm^{-1} (protoheme IX) and 500 cm^{-1} (sperm whale myoglobin). Experimental conditions: Fig. 3 - Hemin•CO, 1 μm, pH 8.0, Ag sol, excitation wavelength at 406.7 nm

Fig. 4 - Mb•Co, 1 μm, pH 8.0, Ag sol, excitation wavelength at 406.7 nm





(d) Detection of Ni-N_ε(His) and Mn-N_ε(His) Vibrations in Hemoproteins.

In collaboration with Dr. John A. Shelnutt et al., we have identified the Ni-N_ε(His) stretch at 241 cm^{-1} in Ni-substituted Mb and at 236 cm^{-1} in Ni-substituted Hb.

Recent experiments on Mn-substituted CTT III and CTT IV revealed a very characteristic line at 213 cm^{-1} which is tentatively assigned as the Mn-N_ε(His) stretch in the Mn(II)Mb species. Further experiments on Mn porphyrins are being carried out to definitely identify this line as the Mn- N_ε(His) mode.

3. Specific Objective for the Coming Year:

(i) To initiate studies on the effects of distal steric hindrance on ν(Mn-NO) stretch and δ(Mn-N-O) bending vibrations in Mn-substituted "Strapped Hemes" (i.e., heme-5, SP-13, SP-14 and SP-15).

(ii) To establish the assignment of Mn(II)-N_ε(Im) stretch in Mn-substituted "picket fence" porphyrin with different bases.

(iii) To resolve the question of whether the ν(O-O) stretch is coupled to the Co porphyrin ring modes by employing ^{15}N-labled Co protoheme IX-substituted hemoglobins (CoCTTII, III or IV).

(iv) To study the vinyl vibrations in pentoheme- and isopentoheme-reconstituted CTTIII complexes.